Applicants and their attorney are aware of U.S. Patent No. 5,013,649 (the "'649" patent), issued to Wang et al. on May 7, 1991, and available as a 102(e) reference against the instant application as of its filing date. In accordance with 37 C.F.R. §1.97, Applicants hereby make a record of the '649 patent. Applicants also make of record U.S. Patent 5,166,058 (the "'058" patent), issued to Wang et al. on November 24, 1992. The '058 patent is not available as a reference but is provided to the Patent Office for completeness and as of interest. PTO Form 1449 and copies of the patents accompany this statement.

The '649 patent focuses on BMP-2A and BMP-2B, two of the four DNA sequences disclosed in WO88/00205, referred to therein as BMP-2 Class I and BMP-2 Class II, respectively. WO 88/00205 is of record in this application. The full length DNA sequences for human BMP-2A and BMP-2B are disclosed in Tables II and III, respectively, of the '649 patent and in Tables VII and VIII, respectively, of WO 88/00205. The '649 patent contains substantially all of the disclosure relating to BMP-2A and BMP-2B in WO 88/00205 and in Wozney et al. (1988) Science 242: 1528-1534 (the "Wozney" article), also of record. In addition, the '649 patent states that a portion of the encoded amino acid sequences may have significance. To wit, the '649 patent states that:

> "BMP-2 proteins are produced by culturing a cell transformed with a cDNA substantially as shown in Table II or Table III and recovering from the culture medium a protein containing substantially the 97 amino acid sequence #299 to #396 of Table II or amino acid #311 to #408 of Table III." (col.1, lines 15-20.)
>
> "The BMP-2A protein encoded by Table II is contemplated to contain the 97 amino acid sequence from amino acid #299 to #396 or a sequence substantially homologous thereto." (col.13, lines 27-31)
>
> "The BMP-2B protein encoded by Table III is contemplated to contain the 97 amino acid sequence

846

from amino acid #311 to #408 or a sequence substantially homologous thereto." (col.16, lines 41-44)

These 97 amino acid sequences, residues 299 to 396 of Table II and residues 311 to 408 of Table III, correspond essentially to residues 4-103 of CBMP-2a and CBMP-2b, respectively, in the instant application (see p. 11 of the specification, for example.) Osteogenically active dimeric proteins comprising the sequences of CBMP-2a and CBMP-2b are nonelected species of the genus claimed in claim 22 in the instant application.

It is respectfully requested that the Examiner review the '649 reference for other pertinent teaching, if any.

Notwithstanding the above, Applicants submit that the '649 patent does not put a species of the protein genus claimed in the instant application into the hands of the public. The genus claimed herein requires that the protein comprise a pair of polypeptide chains "disulfide-bonded to form a dimeric species" having "a conformation capable of inducing cartilage and bone formation in association with a matrix when implanted in a mammal." (Claim 22)

The '649 specification is limited to a description of how BMP-2A and BMP-2B DNA sequences were cloned. Examples are provided for recombinant expression of the full length sequences in COS cells and for testing biological activity in vivo. The example provided for biological activity using recombinantly produced protein fails to induce bone formation. The assay produces only "cartilage-like" nodules at 7 days post implantation (col. 22, lines 49-51). Leaving aside for the moment the issue whether this term describes true cartilage formation or merely the cartilaginous material commonly formed as part of the inflammatory response, we note that the same result

947

("cartilage-like" nodules) was obtained by Wang et al. using BMP-1 (see WO 88/00205, Example VII, pp. 54-55). Wang et al. subsequently speculate BMP-1 is a regulatory protein, possibly a protease which activates osteogenic proteins, (see Wozney, supra, particularly p.1529, col.2, para.3 and p.1533, col.1, para 1.) Applicants are aware of no reports that BMP-1 can induce formation of endochondral bone. In the Wozney article, submitted for publication after the filing date of the '649 patent, in vivo assays performed using recombinant BMP-1, BMP-2 or BMP-3 protein expressed from two different mammalian cell types produced only "islands of cartilage formation" and no activity was seen when BMP-2A or BMP-3 constructs were expressed in a prokaryotic host. Ibid, p.1531, col.2, para. 1. Moreover, it is evident from the discussion in the paper the authors had no confidence that any one of the BMP proteins they identified, acting alone, is a true osteogenic protein, capable of inducing cartilage and bone formation without the assistance of other factors. The authors note:

> "It seems quite likely that the BMP activity derived from bovine bone matrix represents the combined action of multiple factors acting at specific points during bone development. Once significant amounts of purified and active protein from each of our recombinant factors are available...studies can be performed to determine whether the islands of cartilage formation observed in our assay can develop into larger areas of cartilage and bone. While simple mixing of the three BMP proteins may result in this goal, it is more likely that detailed characterization of various subunit compositions and analysis of their modes of action will be necessary to define optimal BMP activity. Thus the configuration and the ratio of these new growth factors may modulate the specificity of the in vivo cartilage or bone-forming activity. It is also possible that additional factors, derived from bone but without inherent bone-forming activity, are necessary to enhance activity of the factors we have described." (Ibid. p. 1532-1533)

The authors go on to suggest that their proteins alone might be useful in cartilage repair but that to induce true

JHP

endochondral bone formation likely would require all their recombinant factors (at least). Specifically, they state:

> "Use of local bone induction to treat large bony defects caused by trauma, surgical refections, or periodontal disease might require all the recombinant human factors necessary for classic BMP activity formulated in an appropriate carrier. For treatment of other types of defects, such as damaged cartilage, a subset of these factors locally delivered may be sufficient." (p. 1532)
>
> "Our findings indicate that the BMP activity in the original preparation is due to a mixture of regulatory molecules and that the complex development process of cartilage and bone formation is most likely controlled, at least in part, by the interactions of these molecules." (p.1529).

In fact, not until July 1989, 25 months after a filing disclosing BMP-2A and BMP-2B DNA (WO88/00205), 15 months after the filing of the '649 patent, and some 5 months after the effective filing date of this application, did Wang et al. apparently discover and disclose that it was possible that properly post-translationally modified BMP-2A alone could induce bone formation in vivo (see U.S. Patent 5,0166,058, the "'058" patent, copy enclosed, filed July 11, 1989, issued November 24, 1992). The '058 patent contains substantially all of the disclosure of the '649 patent, with several notable exceptions, and adds substantially all of the disclosure in Wang et al. (1990) PNAS 87:2220-2224 (the "PNAS" article), which was submitted for publication in December of 1989 and published in March of 1990. The '058 patent differs from the PNAS article in that, while bone formation is asserted in the '058 patent, no data is presented. More significantly, only in the '058 patent and the PNAS article did Wang et al. apparently finally appreciate that BMP2 expression product required appropriate processing of the primary translation sequence to form an osteogenically active material, stating:

249

"Preliminary experiments indicate that over 90% of the biological activity in the total protein pool is eluted from a non-reduced SDS-PAGE at a relative mass of 30,000 daltons. It is contemplated therefore that a dimer of [mature BMP-2A]... accounts for the majority of the biological activity in the mixture of expressed BMP-2A proteins. It is further contemplated that processing of BMP-2A to the mature forms involves dimerization of the proprotein...and removal of the N-terminal region.... ('058 patent, col 26, lines 48-58)."

"We propose that processing of BMP-2A to the 30-KDa form involves dimerization of the proprotein through cysteine(s) in the mature domain...and removal of the N-terminal region...." Wang et al. (1990) PNAS 87:2220-2224 (p. 2222, col. 2, para. 1, lines 6-10).

"...processing of BMP-2A to its active form involves dimerization and cleavage [of the full length primary translated sequence.]" Ibid, p.2224, lines 1-6 of the Discussion)

In the PNAS article the authors go on to speculate that the limited cartilage formation previously detected (e.g., described in the 1988 Wozney article and the '649 patent) may be explained by incomplete processing of the primary translation product which may "affect or even inhibit the biological activity." Ibid, p.2224, lines 18-25 of the Discussion.

Applicants infer from these facts that what may have allowed Wang et al. finally to demonstrate bone formation in the PNAS article submitted in December 1989, and to assert bone formation in the '058 patent, was the use of a different purification scheme for the recombinantly produced material than that disclosed in the '649 patent. This late discovery apparently led to an appreciation of the importance and general nature of the protein structure necessary for biologically active material, (e.g., capable of demonstrable bone formation). Biologically active recombinantly produced material apparently required purification of the expressed protein over a heparin-Sepharose column in the presence of urea, and elution of the bound material

with a linear salt gradient of 0.15-1.0M NaCl, also in the presence of urea. (But see sentence bridging pages 2222-2223 of the PNAS article.) Fractions of the eluted material then were tested for activity and those fractions showing the highest activity were run over a non-reducing SDS-polyacrylamide gel ("SDS-PAGE") and individual protein bands hybridized with BMP-2-specific antisera to identify the presence of BMP-2 protein. (See col. 26-27 of the '058 patent and the PNAS article.) Even so, a minimum of 460 ng (0.46 μg) was required for demonstrable bone formation (see col 27, line 28 of the '058 patent and the PNAS article, p. 2223, col. 2, lines 11-16).

By contrast, the recombinant material described in the '649 patent was partially purified over a heparin-Sepharose column in the absence of urea and eluted in 2M NaCl, again in the absence of urea. Unlike the '058 patent, there is no demonstration in the '649 patent that the material eluted from the heparin-Sepharose column in fact contains any recombinant BMP-2A protein, let alone active BMP-2A. The material tested induced only "cartilage-like nodules", an event also induced by the implantation of non-osteogenic protein. It also is worth noting that, while the '058 patent includes the '649 BMP-2A expression example, missing from the '058 patent are assertions made in the '649 patent that "[p]urified BMP2 proteins are approximately 95% substantially free from other proteinaceous materials." (See col.22, lines 31-33 of the '649 patent.) Also missing are assertions that in vivo assays performed with the "purified" material "indicate that approximately 200 ng of BMP-2A or BMP-2B results on [sic] a score of at least +2." (See col. 22, lines 53-55 of the '649 patent.) From these facts it appears that the '058 patent was filed to correct inadequacies in the '649 patent. This inference is highlighted by the issued claims of the '058 patent which, for the most part, are substantially identical to those of the '649 patent. (MPEP 706.03(y))

Applicants respectfully submit that the disclosure of the '649 patent fails to enable the production of recombinant osteogenic protein comprising the amino acid sequence of CBMP-2a or CBMP-2b which is capable of inducing cartilage and bone formation in a mammal when implanted in the mammal in association with a matrix.

In contrast to the teachings of the '649 and '058 patents, Applicants have consistently pursued elucidation of the osteogenically active form of osteogenic protein and, as early as their first application, filed April 8, 1988 (of which the instant application is a continuation-in-part), understood that the active form of the protein required dimerization and disulfide bonding to produce a dimeric species having the appropriate conformation to induce bone formation in vivo (see U.S. Pat. Nos. 4,698,590 and 5,108,753.) Determination of the sequences and structure necessary for osteogenically active protein allowed Applicants to invent the genus claimed in claim 22 of the instant application. Applicants teach the design and synthesis of osteogenic proteins, means for their recombinant expression, including refolding and oxidation steps to obtain appropriately folded, dimeric species of the recombinantly produced protein, including truncated forms thereof, (see p.67, section E of the specification), and demonstrate both cartilage and bone formation with two exemplary biosynthetic constructs, COP5 and COP7.

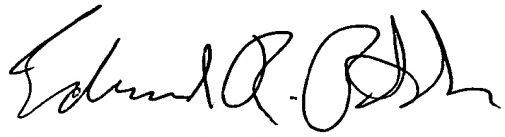
Applicants submit that, with respect to osteogenic proteins, disclosure of a DNA sequence alone, without appreciation of the protein structure and conditions necessary for activity, does not enable persons skilled in the art to produce osteogenically active protein as claimed herein. This is apparent from the fact that Wozney et al. reported osteogenic activity in recombinant material for the first time in the patent application filed July 1989, i.e., 15 months after the filing date of the '649 patent,

252

and 25 months after filing PCT WO 88/00205 disclosing BMP-2A and BMP-2B DNA, and only after Wozney et al. adopted a new purification scheme. Accordingly, the '649 patent does not disclose a species of the claimed genus and fails to anticipate any of the claims as amended of the instant application under 102(e). The claims pending are novel and unobvious over the '649 patent. An immediate notice of allowance is solicited.

If the Examiner believes that a telephone conversation would be helpful in expediting the prosecution of this application, the Examiner is urged to call the undersigned agent at (617) 248-7000.

Respectfully submitted,



Date: December 22, 1992

Edmund R. Pitcher
Attorney for Applicants
Registration No. 27,829

TESTA, HURWITZ & THIBEAULT
Exchange Place, 53 State Street
Boston, MA 02109
(617) 248-7000

299RDK7557/1.AR6

253